"STUDY OF EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN COLORECTAL MALIGNANCIES AND CLINICOPATHOLOGICAL CORRELATION"

Dissertation submitted in partial fulfilment of the requirements for the degree of

M.D. (PATHOLOGY)

BRANCH - III

INSTITUTE OF PATHOLOGY

MADRAS MEDICAL COLLEGE

CHENNAI – 600 003

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

APRIL 2016

CERTIFICATE

This is to certify that this dissertation entitled **"STUDY OF EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN COLORECTAL MALIGNANCIES AND THEIR CLINICOPATHOLGICAL CORRELATION"** is the bonafide original Work of **Dr.K.ASHWINI,** in partial fulfillment of the requirements for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr..M.G.R Medical University to be held in April 2016.

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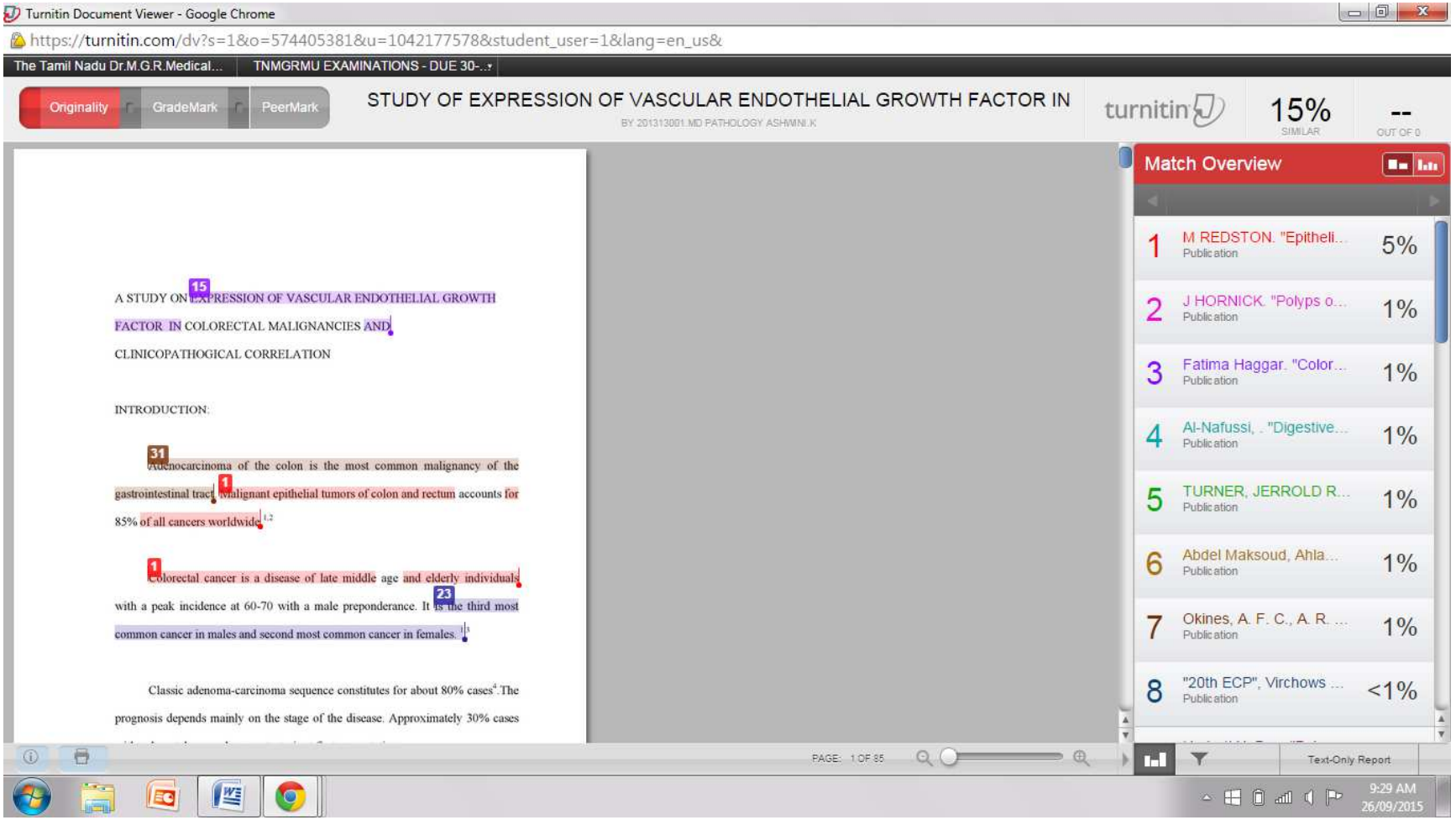
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A STUDY ON EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN COLORECTAL MALIGNANCIES AND CLINICOPATHOGICAL CORRELATION
INTRODUCTION:
Adenocarcinoma of the colon is the most common malignancy of the gastrointestinal tract. Malignant epithelial tumors of colon and rectum accounts for 85% of all cancers worldwide.
Colorectal cancer is a disease of late middle age and elderly individuals with a peak incidence at 60-70 with a male preponderance. It is the third most common cancer in males and second most common cancer in females.
Classic adenoma-carcinoma sequence constitutes for about 80% cases. The prognosis depends mainly on the stage of the disease. Approximately 30% cases
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CERTIFICATE OF APPROVAL

To
Dr. K.Ashwini,
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Dear Dr.K.Ashwini,

The Institutional Ethics Committee has considered your request and approved your study titled **"A study of expression of Vascular Endothelial Growth Factor (VEGF) in colorectal malignancies". No.12102014.**

The following members of Ethics Committee were present in the meeting held on 07.10.2014 conducted at Madras Medical College, Chennai-3.

1. Dr.C.Rajendran, M.D.,	: Chairperson
2. Dr.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
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10. Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

DECLARATION

I, **Dr.K.ASHWINI**, solemnly declare that the dissertation titled **"STUDY OF EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN COLORECTAL MALIGNANCIES AND THEIR CLINICOPATHOLOGICAL CORRELATION"** is the bonafide work done by me at Institute of Pathology and Electron Microscopy, Madras Medical College under the expert guidance and supervision of **Prof.Dr.PADMAVATHI, M.D.,** Professor of Pathology, Institute of Pathology, Madras Medical College. This dissertation is submitted to the Tamilnadu Dr. M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place: Chennai

Date: **Dr.K.ASHWINI**

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The patients, who form the most integral part of the work, were always kind and co-operative. I pray for their speedy recovery and place this study as a tribute to them.

My family, friends and fellow postgraduates have stood by me during my times of need.

Above all, I thank the Lord Almighty for His kindness and blessings without which this study would not have materialized.

ABBREVIATIONS

VEGF	-	Vascular Endothelial Growth Factor
FAP	-	Familial Adenomatous Polyposis
APC	-	Adenomatous Polyposis Coli
HNPCC	-	Hereditary Non Polyposis Colon Cancer
MSI	-	MicroSatellite Instability
MMR	-	MisMatch Repair
WHO	-	World Health Organisation
AJCC	-	American Joint Committee on Colon Cancer
CEA	-	Carcino Embryonic Antigen
CK	-	CytoKeratin
GIST	-	GastroIntestinal Stromal Tumor
FFPE	-	Formalin Fixed Paraffin Embedded
NSAIDs	-	Non Steroidal Anti-Inflammatory Drugs
IEL	-	IntraEpithelial Lymphocytes
COX	-	CycloOXygenase

CONTENTS

ABSTRACT

INTRODUCTION:

Adenocarcinoma of the colon is the most common malignancy of the gastrointestinal tract. VEGF is an important regulator of tumor angiogenesis and is associated with metastasis and poor prognosis[5]. Thus, assessing VEGF expression is colorectal cancer can help in determining the prognosis. In this study, an attempt has been made to study the expression of VEGF in colorectal malignancies, and to compare it with various clinicopathological parameters.

AIMS & OBJECTIVES

- To study the epidemiological aspects of colorectal cancer in patients attending Rajiv Gandhi Government General Hospital from July 2013 – July 2015.
- To assess the expression of VEGF in colorectal cancer.

To compare with the clinicopathological parameters and to assess the prognostic significance.

MATERIALS AND METHODS:

We received 147 cases of resected specimens of colorectal carcinomas, during the period between July 2013 to July 2015.

INTRODUCTION

Adenocarcinoma of the colon is the most common malignancy of the gastrointestinal tract. Malignant epithelial tumors of colon and rectum accounts for 85% of all cancers worldwide.[1,2]

Colorectal cancer is a disease of late middle age and elderly individuals with a peak incidence at 60-70 with a male preponderance. It is the third most common cancer in males and second most common cancer in females. [1,3]

Classic adenoma-carcinoma sequence constitutes for about 80% cases[4].The prognosis depends mainly on the stage of the disease. Approximately 30% cases with colorectal cancer have metastasis at the time of first presentation.

Tumors require neovascularisation for growth and metastasis. VEGF is an important regulator of tumor angiogenesis and is associated with metastasis and poor prognosis[5]. VEGF induces vascular permeability and angiogenesis. Adenomas do not express VEGF.

Thus, assessing VEGF expression is colorectal cancer can help in determining the prognosis. Therapies targeted on VEGF receptors can improve

4um thick sections were taken and stained with hematoxylin and eosin. 50 random cases were selected for immunohistochemical studies with VEGF.

RESULTS:

- The median age at presentation is 60 years
- Maximum number of cases occurred in the age group of 50-60 years
- There is a male preponderance -85 cases (57.8%)
- Left sided tumors are more common-104 cases (70.7%)
- 54% cases had tumors less than 5 cm in greatest dimension
- Commonest gross appearance is ulceroproliferative-92 cases (62.6%) followed by the ulcerative type-23 cases(15.6%)
- Most common histopathological subtype is Infiltrating adenocarcinoma-123 cases (83.7%).
- Commonest grade is moderately differentiated-95 cases (64.6%)
- 53 cases(36.1%) belonged to Astler-Coller stage C2.
- 66 cases(44.9%) had lymph node involvement
- 74 cases(50.3%) had lymphatic invasion
- 67 cases(45.6%) had vascular invasion
- 76 cases(51.7%) had tumor infiltrating lymphocytes
- Resected margins were free in 147(97.3%) cases

- Out of 147 cases, immunohistochemical analysis was done for expression of VEGF in 50 cases.
- 9(18%), 20(40%) and 21(42%) cases had 1+,2+ and 3+ levels of VEGF expression respectively
- Statistically significant association is present between VEGF expression and factors like presence of lymphatic invasion, vascular invasion, lymph node metastasis and stage of the tumor.

KEY WORDS:

Vascular Endothelial Growth Factor(VEGF), Neovascularisation, Immunohistochemistry

survival. VEGF expression in the cytoplasm can be determined immunohistochemically by using monoclonal antibodies.

In this study, an attempt has been made to study the expression of VEGF in colorectal malignancies, and to compare it with various clinicopathological parameters.

AIMS & OBJECTIVES

- To study the epidemiological aspects of colorectal cancer in patients attending Rajiv Gandhi Government General Hospital from July 2013 – July 2015.

- To assess the expression of VEGF in colorectal cancer.

- To compare with the clinicopathological parameters and to assess the prognostic significance.

REVIEW OF LITERATURE

INTRODUCTION:

Colorectal carcinoma is one of the common malignancy of the gastrointestinal tract. It has a peak age incidence of 60-70 years. The classic adenoma-carcinoma sequence accounts for about 80% cases. About 30% patients diagnosed with colorectal carcinoma have regional or distant metastasis at the time of first presentation.

Tumors require neovascularisation for growth and metastasis. VEGF is an important regulator of tumor angiogenesis and promotes regional and distant metastasis, thereby reducing survival.

VEGF can induce vasculogenesis and lymphangiogenesis. Adenomas do not express VEGF. Thereby assessing the expression of VEGF in colorectal malignancies can help in determining the prognosis and in improving patient survival.

EPIDEMIOLOGY:

Colorectal cancer is an important public health problem and a major cause of morbidity and mortality throughout the world[6]. There is a large geographic difference in global distribution of colorectal cancer. Countries with high risk include Australia, New Zealand, Canada and those with low risk include China, India, Africa and South America[7].

Global age standardized rates of colorectal carcinoma incidence are higher in men than in women (19.1/1,00,000 in men and 14.4/1,00,000 in women)[8]

Of all the cases reported worldwide, incidence in developed world accounts for about 63% of cases[9]. Incidence rates varies upto 10 fold between countries with the highest rates and those with lowest rates[10,11]. Most colorectal cancers arise from benign adenomatous polyps lining the wall of the bowel[12]. Development of colorectal cancer is a multistep process[13].

INCIDENCE IN INDIA

In India, the annual incidence rates for colon and rectal cancer in men are 4.4 and 4.1/100000 respectively. The AAR for colon cancer in women is 3.9/100000.Colon cancer ranks eighth and rectal cancer ranks ninth among men. In women, rectal cancer does not figure in top ten, whereas colon cancer ranks ninth[14].

MORTALITY

Colorectal cancer is the fourth most common cause of death from cancer worldwide[15]. Survival is highly dependant on stage of the disease at diagnosis and ranges from 90% five years survival for cancers detected at the earlier stage, 70% in patients with regional metastasis and 10% in patients diagnosed with distant metastasis[16,17].

ETIOLOGY AND RISK FACTORS

The risk factors can be classified as genetic, environmental, life style related factors.

Genetic Factors

Colorectal carcinomas may or may not be associated with colonic polyposis. Colonic polyposis syndrome includes:

- Familial adenomatous polyposis and its variants like Turcot syndrome, Gardner syndrome and attenuated FAP

- Hereditary non polyposis colon cancer or Lynch syndrome comprises non-polyposis category.

- Familial adenomatous polyposis is characterized by multiple colonic adenomatous polyps appearing in childhood with subsequent transformation to malignancy at an average age of 45 years and it is caused by genetic mutation in APC gene on chromosome 5[18].

- Turcot syndrome- a variant of Familial adenomatous polyposis, associated with multiple colorectal adenomas and primary neuroepithelial brain tumors.

Germline mutations of mismatch repair genes have been demonstrated.

Gardner syndrome- includes mandibulomaxillary osteomas, multiple epidermoid cysts and multiple colonic polyps.

- Attenuated FAP is associated with the same genetic mutation as in FAP, but is characterized fewer adenomas and later age of colorectal cancer presentation.

- MYH-associated polyposis is inherited in an autosomal recessive pattern. Mutations in the base excision repair gene mut Y homologue has been demonstrated.

Hereditary Non-Polyposis Colon Cancer (Lynch syndrome):

It is an autosomal dominant condition. Defects in any one of the following mismatch repair genes have been demonstrated- MLH 1, MSH 2, PMS 2 or h MSH 6.

ENVIRONMENTAL FACTORS:

Age – Old age has high risk

Gender – Colorectal cancer has a male preponderance[19]

Inflammatory Bowel Disease – Both ulcerative colitis and Crohn's disease can cause colorectal cancer[20]. Extent of the disease, duration and activity are primary determinants[21]. People with Crohn's disease have three times more risk of developing colorectal cancer compared to the general population[22].

Ureterocolic anastomosis, long term immunosuppression[23], following organ transplantation, insulin resistant Diabetes Mellitus (due to long-term effects of insulin-like growth factor)[24,25], pelvic irradiation[26] increase the risk of colorectal carcinoma.

LIFESTYLE RELATED FACTORS:

Consumption of fresh red meat and processed meat is associated with increased risk[27,28,29]. Alcohol consumption is a risk factor and reduction in alcohol consumption may reduce the incidence of colorectal cancer, especially in those with a positive family history[30].

There is an inverse association with vegetable and fibre consumption[31,32]. Obesity[33], cigarette smoking [34], sedentary lifestyle[35], increase the risk. Folate ingestion reduces the risk[36]. Use of NSAIDS, especially daily ingestion of low-dose aspirin decreases the incidence of colorectal cancer[37].

COX-2 is overexpressed in 90% colorectal carcinomas and 40-90% adenomas. COX-2 is essential for prostaglandin E2 synthesis which in turn leads to epithelial proliferation, especially after epithelial injury. N-SAIDS inhibit COX-2, thereby reducing PGE-2 production and incidence of colorectal carcinoma[38].

ANATOMY, EMBRYOLOGY & PHYSIOLOGY

It is necessary to study the difference between the segments of the large bowel to understand the mechanism of colorectal cancer.

The large intestine extends from the distal end of ileum to anus and measures about 1.5m; It consists of caecum, ascending colon, transverse colon, descending colon, sigmoid colon and rectum.

The junction between ascending colon and transverse colon is called hepatic flexure and the junction between transverse colon and descending flexure is known as splenic flexure. Rectum measures about 8-15 cm, lies in the pelvis and ends at the anal canal.

General characteristics of the large intestine are its large internal diameter compared to that of the small intestine and the presence of omental appendices which are peritoneum covered accumulations of fat.

The longitudinal muscle layer is segregated into 3 narrow bands called taeniae coli, which are prominent in the caecum and colon, less visible in the rectum. Haustrations represent the sacculations of the colon. Mucosa of the colon appears flat as there are no villi. Numerous non-branching crypts are seen punctuating the mucosa.

The proximal and distal colon are intraperitoneal whereas rectum is retroperitoneal. Proximal colon embryologically develops from the midgut, nourished by the branches of superior mesenteric artery, innervated by the

vagus nerve. The capillary network of the proximal colon are multilayered with increased capillary width in order to absorb water and electrolytes[39,40].

Distal colon develops from the hindgut, nourished by the branches of inferior mesenteric artery, innervated by S2-S4 nerves. The capillary network is single-layered. Wall of the colon is thinner when compared to rectum when visualized by endoscopic ultrasonography with a higher average crypt length[41].

Histologically, the bowel wall consists of four layers namely mucosa, submucosa, muscularis proproia or muscularis externa and serosa.

The mucosal surface is lined by a single layer of columnar cells. The surface epithelium is composed of absorptive cells and mucin secreting goblet cells. The epithelial cells rests on a thin basement membrane.

The crypts of Lieberkuhn open directly on to the surface. The crypts have a test-tube like shape and are arranged parallel to each other.

Immunohistochemically, the epithelial cells of the normal colonic mucosa contain CK 18,CK 19, CK 20 but not CK 7.

Lamina propria consists of collagen fibres, vessels, nerves, smooth muscle bundles, few lymphocytes, plasma cells and histiocytes.

The submucosa contains loose connective tissue with vessels and nerves. Submucosal layer contains Meissner’s plexus of nerves.

Muscularis propria consists of inner circular and outer longitudinal layers. Auerbach's myenteric plexus of nerves are present in between these two muscular layers.

Serosa is composed of single layer of flattened mesothelial cells. Mucin secreting goblet cells are higher in rectum and sigmoid colon and there is a high concentration of endocrine cells in the rectum. In descending colon, neutral mucin is predominant, whereas in rectum acidic mucins predominate[42].

Sites of colorectal malignancies

Rectum and sigmoid colon are the most common sites for colorectal carcinomas. In recent years, there is an increase in the incidence of right sided colonic cancers. (arising proximal to splenic flexure)[43,44].

Females, past history of cholecystectomy, hormone therapy for prostate cancer, multiparity are some of the factors increasing the risk, possibly due to a change in metabolism of bile acids either in the component or quality.

PATHOGENESIS OF COLORECTAL CARCINOMAS

Most colorectal carcinomas arise from adenomas[45]. Residual adenoma can be identified in about 10-30% cases while the remainder are overgrown and the precursor lesion is not apparent histologically[46]. Adenomas precede cancer by 15 years[47].

ABERRANT CRYPT FOCI:

It is the earliest morphological precursor of epithelial neoplasia. Aberrant crypt foci have crypts of enlarged caliber and thickened epithelium with mucin depletion[48]. There are two subtypes:

1) Hyperplastic type with RAS protooncogene mutation.
2) Dysplastic type with APC gene mutation

They represent the intermediate step between normal colonic epithelium and grossly apparent adenomatous growth[49].

Genetic Model:

Accumulation of genetic alterations leads to progression of adenoma to carcinoma[50,51,52].

Genome Instability:

It refers to increased acquisition and tolerance of mutation by the cells – which is a hallmark of colorectal cancer development[53,54].

Genomic Instability is subdivided into:

- Chromosome instability
- Microsatellite instability –

  accounting for 85% and 15% colorectal carcinomas respectively.

APC was first identified as the gene mutated in FAP[55].

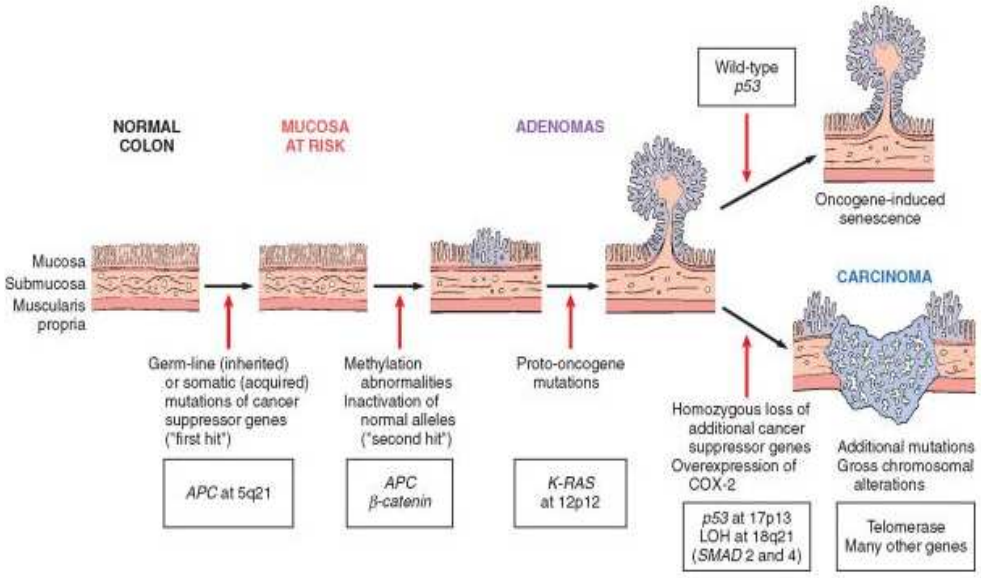
Sporadic colorectal cancers can also harbor APC mutation[56].

Thus APC is known as the gatekeeper gene of colorectal neoplasia.

Other genetic alterations in colorectal carcinoma are the presence of activating KRAS mutations in about 35% of cases, leading to uncontrolled growth and reduced apoptosis[57]. Due to chromosomal deletions, TP53 inactivation occurs is 50-70% cases[58].

ADENOMA-CARCINOMA SEQUENCE:

It accounts for 80% of sporadic colon tumors.



Molecular basis for the evolution of colorectal cancer through the adenoma- carcinoma sequence.

APC-Beta-catenin pathway:

Both mutation and epigenetic events like methylation induced gene silencing can cause functional inactivation of both copies of APC gene.

APC is a negative regulator of beta-catenin, a component of WNT-signaling pathway. The function of APC is to bind and cause degradation of beta-catenin.

With loss of APC function there is accumulation of beta-catenin which translocates to nucleus and causes transcription of genes encoding MYC and Cyclin D, thereby promoting proliferation.

Thus, in APC/B-Catenin pathway, chromosomal instability is the hallmark.

Microsatellite Instability:

Mutations accumulate in microsatellite repeats, in patients with DNA mismatch repair deficiency and this is referred to as microsatellite instability. When these mutations involve the coding / promoter region of the gene responsible for reputation of cell growth, uncontrolled cell growth occurs and increased survival of genetically abnormal clones ensures.

BRAF mutations can also occurs.

Combination of MSI, BRAF mutation and methylation of specific targets are the hallmark o this pathway[59]. The reference standard for large

intestinal cancers in a panel of 5 microsatellites (BAT 25, BAT 26, D5S346, D2S123, D17S250)[60.]

When instability occurs in any of the 2 microsatellites, it is called MSI-H and if instability occurs in only one microsatellite it is called MSI-L.

Genetic Susceptibility:

Colorectal carcinomas can be associated with polyposis syndromes and non-polyposis syndromes.

ADENOMATOUS POLYPOSIS SYNDROMES

Familial Adenomatous Polyposis:

It is an autosomal dominant condition. Mutated APC gene can be inherited or new germline mutation of APC can occur[61]. Adenomas occur as a result of loss of second APC allele within colonic epithelial cells[62].

FAP is defined as the presence of >100 adenomatous polyps in the colon, many patients have several hundreds to thousands of polyps. Adenocarcinomas occurs in mid-30s and as early as 17 years.

FAP accounts for about 1% of colon cancers. There is equal sex predeliction and the frequency is about 1 in 8000 – 14000 in general population[63]. In FAP, COX2 inhibitors play a role in reducing polyp burden[64].

Subtypes:

Gardner syndrome, Turcot syndrome, attenuated FAP. Treatment includes screening during adolescence and post adolescent prophylactic colectomy.

Gardner's Syndrome:

Patients with FAP who have manifestations in addition to those in GI tract are said to have Gardner's syndrome[65,66,67.]

Clinical features include epidermal cysts, dental abnormalities, osteomas of mandible, skull and bony bones, aggressive desmoid tumors and congenital hypertrophy of retinal pigment epithelium.

Turcot Syndrome:

It refers to the coexistence of hereditary colon cancer syndrome (FAP / HNPCC) and CNS Tumors[68,69], most commonly medulloblastoma, astrocytoma and ependymoma. Distinct germline defects in either APC (in FAP) / DNA mismatch repair genes (in HNPCC).

Attenuated FAP:

Polyps are less than 100 in number (usually <30)[70,71]. Adenomas and adenocarcinomas develop in later stages in life than in classic FAP. Lifetime risk of developing colonic malignancy is 80%. Flat adenomas are common.

HNPCC / Lynch syndrome:

It has autosomal dominant mode of inheritance.

Inherited defects in atleast one of the family of DNA mismatch repair enzymes are present (hMLH, hMSH2, hMSH6, hPMS2)[72,73] which leads to microsatellite instability and rapid accumulation of somatic mutations in genes that control tumor progression. Risk of developing colon cancer is 80-90%.

Revised Bethesda guidelines for diagnosing HNPCC is given in annexure I.

HNPCC accounts for about 5% of all colonic cancers, with a predominance of right sided carcinomas at an early age. The tumors have signet-ring / mucinous component with a microglandular or medullary growth pattern with pushing margins. They are usually poorly differentiated with prominent tumor infiltrating lymphocytes.

Juvenile polyposis syndrome:

It is characterized by the presence of multiple juvenile polyps throughout the bowel. Juvenile polyps are also called retention polyps. Microscopically, juvenile polyps contain ulcerated surface with granulation tissue formation and underlying cystically dilated glands filled with mucus and separated by an edematous stroma.

This condition is associated with development of multiple adenomatous polyps, leading to adenocarcinoma.

Inactivating mutations of SMAD 4 have been demonstrated.

Cronkhite – Canada syndrome:

It is a non- hereditary disorder characterized by multiple juvenile polyps associated with ectodermal changes. Adenomatous polyps and adenocarcinoma can develop.

Peutz- Jeghers syndrome:

It is characterized by the presence of Peutz – Jegher's polyps. These polyps are defined by the presence of ramifying smooth muscle fibres from the muscularis mucosa, amomg the glands.

Germline mutations of LKB 1 gene have been demonstrated. This syndrome can be assoiated with adenomatous polyps with high grade dysplasia and adenocarcinoma of the large bowel.

Cowden syndrome:

It is an autosomal dominant condition, also known as multiple hamartoma syndrome . the polyps are characterized by disorganization and proliferation of the muscularis mucosa.

Torre- Muir syndrome:

It is an autosomal dominant condition. Around 15% of females with this condition develop endometrial cancer and 50% of individuals with this syndrome develop colorectal carcinoma, most of which are found in the right colon.

NOTE: Any polyposis syndrome involving large intestine can evolve into a malignancy, FAP and Grdner syndrome having the greatest risk.

GROSS APPEARANCE:

The gross appearance of colorectal carcinoma depends strongly on stage of the disease at diagnosis. ‘Small lesions’ may be sessile or pedunculated. ‘Large’ carcinomas can be classified into 4 subtypes.

1) Exophytic or polypoid tumors – rarely they cause obstruction and occur commonly in the caecum.

2) Annular or constricting tumors – proximal fragment is dilated with flattened mucosa. It produces a ‘apple core appearance’ on radiography commonly causes functional obstruction.

3) Infiltrative and ulcerating tumors – they are often raised, with irregular edges and central excavated area infiltrating into deep layers of bowel wall.

4) Diffuse tumors – this subtype is similar to linitis plastica of stomach, with diffuse flattening and thickening of the colon.

Cut section of the tumor appears homogenous admixed with areas of necrosis. Dilatation can occur as a result of obstruction and retraction of serosa occurs due to invasion of tumor into muscularis propria or subserosa.

MICROSCOPIC APPEARANCE:

Criteria for malignancy:

Intramucosal adenocarcinoma refers to the malignancy confined to the lamina propria and muscularis mucosa. It is almost never associated with lymph node metastasis[74] due to the relative paucity of lymphatics. Intramucosal carcinomas are denoted as Tis. Therefore, invasion into submucosa is required to call the colorectal carcinomas as T1.

Diagnosis by Biopsy:

Endoscopic biopsies are used for diagnosis. To determine the presence of invasion is the most important aspect of pathological examination. **WHO classification of tumors of colon and rectum are given in annexure II.**

Adenocarcinoma:

Colonic adenocarcinoma are usually moderately differentiated, the tumor cells being arranged as medium to large sized glands with moderate variability in their size and configuration with a moderate amount of stroma.

Well differentiated tumors contain tall and columnar epithelial cells. The cells become polygonal or cuboidal with decreasing degrees of differentiation. Numerous mitotic figures are present.

Dirty Necrosis:

Refers to the presence of inspissated eosinophilic mucus, nuclear and cellular debris within the glandular lumen. Thus, when dirty necrosis is present

in a metastasis from an unknown primary, it is worth to search for a colorectal primary.

Leading edge of the tumor is usually associated with infiltrating glands.

Desmoplastic reaction in the stroma can be prominent the presence of other cells in variable amounts, for example, Paneth cells, neuroendocrine cells, squamous cells, trophoblasts are of no prognostic significance.

Grading:

Grading is primarily based on the population of the tumor cells that is composed of glands when compared to the areas with solid nests or cords of cells with lumina. Grading system endorsed by AJCC and WHO are used commonly[74,75].

The tumors are graded according to the amount of differentiation.

When >95% of the tumor cells are arranged as glands, they are said to be well differentiated.

When <5% tumor cells are arranged as glands, they are called poorly differentiated tumors.

When there is no apparent gland formation; they are known as undifferentiated tumors.

According to this grading system, well, moderate and poorly differentiated tumors amount to 10%, 70%, and 20% cases respectively.

The diagnosis of poorly differentiated adenocarcinoma has the highest rate of reproducibility and it is the one with a poor survival rate.

Mucinous adeocarcinoma:

The term mucinous carcinoma refers to the tumors composed of >50% extracellular mucin. When the mucinous component is >10% or <50%, the tumors are referred to as adenocarcinoma with mucinous differentiation.

Mucinous carcinomas contain strips of epithelial cells floating in extracellular pools of mucin and a variable member of signet ring cells may also be present. They constitute about 10% of all colonic cancers. They are common in patients with HNPCC and tend to present at a late stage. Microsatellite instability and defects in DNA mismatch repair are common. [76]

Grossly, they are exophytic. Cut surface is soft and gelatinous. Paucity of fibrous tissue imparts a ‘colloid’ appearance to the cut surface.

Expression of HATH1, a transcription factor in activation of MUC2 in colonic epithelium is a possible biologic basis for mucinous tumors. Mucinous carcinomas account for a greater proportion of right sided colonic tumors.

Microscopically, mucinous adenocarcinomas have abundant large glandular structures embedded in extracellular pools of mucin. The mucin shows positive staining with Alcian blue or PAS stains.

The tumor has infiltrative margins which contributes to the poor prognosis.[77,78,79]

They are more likely to develop peritoneal implants[80] and a propensity to invade adjacent viscera[81] and involve lymph nodes beyond the pericolonic region.

Signet ring cell adenocarcinoma

They constitute about 0.5-1% of colorectal carcinomas and contains atleast 50% signet ring cells. They have a slight male preponderance and mean age of occurrence is 64 years.[82]

IBD is a risk factor for this tumor.[83] They occur in equal frequency in right and left colon. Grossly, they are ulcerative. Microscopically, the tumor cells contain a characteristic mucin vacuole, which pushes the nuclei to the periphery of the cytoplasm.

The mucin in MUC-2 positive.[84] The patients present at a later stage[85]. Peritoneal seeding is common 5 year survival rate is less than 10%.

Undifferentiated carcinomas

These tumors contain evidence of epithelial differentiation but there is no obvious gland formation there is less than 5% gland formation.

Grossly, they are bulky due to increased cellularity and soft due to lack of desmoplasia, contain extensive areas of necrosis.

Microscopically, they have an infiltrating growth pattern. The tumor cells are arranged as sheets, cords and trabeculae with variable degrees of anaplasia.

Medullary carcinoma

Also known as large cell minimally differentiated carcinomas[86]. It was first described by Gessures and co-workers[87].

The tumor cells have abundant cytoplasm, vesicular nuclei and prominent nucleoli and is associated with marked tumor infiltrating lymphocyte response. These tumors are common in women, caecum or proximal colon and associated with DNA mismatch repair.

They are negative for CK20 and positive for CK7. They have a favourable outcome.

Adenosquamous carcinoma

Constitute about 0.06% of all colorectal cancer. This tumor is associated with paraneoplastic hypercalcemia and PTH rP[88,89]. IBD is a risk factor [90,91]. They occur in equal proportion is right and left colon. Overall 5 years survival rate is 3.1%[92].

Squamous Cell Carcinoma

It accounts of 0.1% of are colorectal cancer cases probable histogenesis is from a pluripotent stem cell [93,94] capable of multidirectional differentiation. HPV plays a role in pathogenesis[95].

Criteria for diagnosing primary SCC in colon

- Exclusion of metastasis from other sites.
- Extension from carcinoma of anus.
- An associated squamous – lined fistulous tract must be excluded.

Micropapillary carcinoma

It is an uncommon variant with a aggressive behavior. Microscopically, the neoplastic cells are arranged as balls or clusters and the cells have eosinophilic cytoplasm and vesicular nuclei. The tumor cells are surrounded by cleft-like spaces.

The micropapillary component ranges from 5-80%. This variant is associated with a higher incidence of nodal and distant metastasis.

The micropapillary pattern can be mistaken for tumor budding. But the tumor cell nests of micropapillary carcinoma are larger than that of budding[96,97].

Small Cell Variant

Constitutes <1% of all colorectal cancer.[98] Microscopically they are similar to small cell carcinoma of lung. One third of these cases arise from typical adenomas.

Areas of squamous differentiation may be present. Expresses NSE, synaptophysin, chromagranin and has an extremely poor prognosis.

Serrated Adenocarcinoma

It refers to cancers arising from sessile serrated polyps or serrated adenomas.[99] About 7.5% of all colon cancers are associated with serrated precursor lesions [100].

Characteristic features of serrated carcinomas include serration of the glandular lining epithelium, cells with abundant eosinophilic cytoplasm and nuclei with peripheral condensation of chromatin.

Subtypes include;

- Proximal MSI-H cancers arising from sessile serrated polyps.
- Distal MSI-H cancers arising from serrated adenomas.

Other Variants:

Carcinomas with spindle cell component are called sarcomatoid carcinoma. The spindle cells are immunoreactive for keratin.

Carcinosarcoma refers to malignant tumors with both carcinomatous and heterologous mesenchymal elements.

Rare hsitopathological variants of colorectal carcinoma includes pigmented, clear cell, pleomorphic, paneth cell rich variants.

Colorectal cancer in patients younger than 40 years

It accounts for 1-2% of all colorectal cancers. A predisposing factor is present in about 21% of cases [101]. The patients present at an advanced stage, most are <40 years and have regional or distant metastasis at the time of diagnosis. [102,103]

Common histological variants includes the mucinous adenocarcinoma and signet ring cell type tumors. Prognosis is poor in these cases.

IMMUNOPHENOTYPE

It is used to differentiate between primary and secondary tumors and for the characterization of several subtypes.

CEA is the most commonly used stain to identify primary colonic cancer.

CK-7 and CK-20 can also be used.

Villin is a protein that identifies intestinal differentiation.[104]

CDX2, transcriptional factor is expressed in normal crypt epithelium and in 90% of colorectal adenocarcinomas [105,106]. There is an immense relationship between tumor stage and CDX2 expression.

MUC-2 is also a specific marker for colorectal carcinoma.

COLORECTAL CANCER SCREENING:

It is done using a number of tests like stool examination for occult blood, colonoscopy, flexible sigmoidoscopy, radiological tests comprising of double contrast barium enema and CT colonography.

Screening for colorectal cancer is recommended for men and women more than 40 years of age as part of their routine annual check-up.

PROGNOSIS:

5 year survival rate for colorectal carcinoma, after curative resection is 40-60% [107,108,109]. Two thirds of regional lymph node metastasis or local recurrences are evident within first 2 years[110].

PROGNOSTIC FACTORS:

Age:

Very young age and very old age are associated with poor prognosis.[111]

Gender:

Females have better prognosis.

Tumor location:

Tumors of left colon have a favourable outcome while those arising in sigmoid colon and rectum have a worse outcome[112].

Serum CEA levels:

Serum CEA levels >5ug/ml has a adverse effect on prognosis [113,114].

Local Extent:

Microscopic carcinomas found incidentally in a polyps has excellent prognosis. Tumors extending beyond the bowel wall have worse prognosis.

Tumor Size:

Size of the tumor correlates with prognosis [115] and does not have any relation with metastasis to regional lymph nodes.[116]

Tumor edge:

Tumors with non-polypoidal edges have worst prognosis.[117,118]

Obstruction:

Tumors presenting with destruction have a poor prognosis[119,120].

Perforation:

Extensive bowel wall infiltration by the tumor leads to perforation, which has got a poor prognosis[121].

Tumor Margins:

Carcinomas with pushing margins have a better prognosis.[122,123]

Tumor budding:

It is defined as the presence of isolated tumor cells or dusters of more than five cells at the invasive edge of the tumor, seen to migrate into the desmoplastic stroma.[124] It has got a poor outcome.[125,126]

Vascular invasion:

It is a sign of significant increase in incidence of distant metastasis.

Extramural venous invasion is an independent prognostic factor.[127,128]

Angiogenesis:

Increased tumor angiogenesis predicts recurrence and is associated with reduced survival. Perineural invasion, when present is a poor prognostic sign.

Lymphnode involvement:

Adequacy of pathological examination is evaluated by the total number of lymph nodes sampled.[129] Lymph node metastasis away from the primary tumor is usually associated with poor outcome.

Micrometastasis refers to solitary lymph node metastasis <2mm in size.[130,131]

SPECIAL TECHNIQUES TO IMPROVE LYMPH NODE DISSECTION:

Fat clearance methods:

Fat clearance methods of lymph node dissection by immersing the tissue in graded alcohol solutions can be used to increase the yield of lymph nodes. But, this method should be used only after complete assessment of the circumferential margins, the status of which is an extremely important prognostic factor.

Sentinel lymph node examination:

They are the nodes which have the most direct drainage from the tumor. Therefore, meticulous examination of the sentinel lymph nodes can help in identifying patients who have primary tumor confined to the bowel wall and having unidentifiable metastasis at the time of surgical resection.

Sentinel lymph nodes are identified by injecting a blue dye in the subserosal layer. One to four lymph nodes that change color first are considered as sentinel lymph nodes.

Host lymphatic response:

Various immunological and inflammatory reactions occur in response to colorectal cancer, including peritumoral lymphocytes, tumor infiltrating lymphocytes, reactive hyperplasia of regional lymph nodes etc.

Crohn's like lymphoid reaction in the peritumoral region can be graded as none, mild and marked. It is an independent prognostic factor.[132,133]

Tumor infiltrating lymphocytes are associated with colorectal cancers containing DNA mistmatch repair deficiency.[134] 5-7 IELS/HPF is the cut off and it is a specific and sensitive marker for identification of MSI-H cancers.

Microscopic tumor types:

Mucinous carcinoma, signet ring cell carcinoma are variants with poor prognosis.

Staging:

Pathological staging is the most important factor determining the tumor behavior and patient outcome.[135]

AJCC and modified Astler-Coller staging are given in annexure III. Pericolonic tumor deposits, when present is a poor prognostic factor.[136]

Margin Status:

The presence of tumor in the radial margin is the most important factor in predicting local recurrence.[137,138]

Tumor thickness:

The thickness of tumor in the central depressed correlates with presence of lymph node and liver metastasis. [139]

Liver metastasis:

About 15-25% cases have liver metastasis at the time of presentation; 20% patients develop metastasis after treatment of primary tumor[140]. Without treatment, median survival after detection of liver metastasis is about 9 months.[141]

In some patients, 5 year survival rates can be improved with resection of liver metastasis.[142]

After resection, the status of resected margins remains the most important prognostic factor. Patients with negative margins have a better outcome [143]. Clearance of >1cm has a better outcome.[144]

TARGETED THERAPY:

The history of targeted therapy dates back to 1971, when Folkman hypothesized that administration of an agent that prevents angiogenesis can have dramatic effect on cancer treatment.

Targeted therapy is a type of chemotherapy which takes advantages of trivial differences between normal cells and cancer cells. The genetic and protein changes in cells causing cancer have been learnt through various researches. Newer drugs have been developed to target these changes.

Their mechanism of action differs from that of standard chemotherapy drugs and often they have less adverse effects. They can also be used along with the standard chemotherapy regimes.

In colon cancer, monoclonal antibodies against VEGF and EGFR have been used for targeted therapies.

Bevacizumab is a well-known drug that inhibits angiogenesis. It was first approved based on its ability to improve survival in colorectal cancer patients.

When used in conjunction with cytotoxic chemotherapy, it led to an additive suppression of tumor cell growth by enhancing apoptosis.

Many other anti-angiogenesis agents like Valatinib, Afibercept are under trial.

Many studies have been conducted based on the expression of VEGF in colorectal cancer.

In a study conducted by Bendarafa R et al., in 360 colorectal cancer patients, cytoplasmic VEGF expression in the tumor cells was assessed by automated immunohistochemistry. Significant statistical association was found between VEGF expression and factors like location of the tumor (left sided tumors expressed VEGF more than the right sided tumors), stage of the disease and 10-year disease specific survival. Thus, assessing VEGF expression in

colorectal carcinoma helped in selecting patients who are likely to benefit from neoadjuvant chemotherapy.

Zafirellis et al., did a study in 117 colorectal cancer patients and assessed the cytoplasmic VEGF expression in the tumor cells using semi-automated computerized image analysis. Statistically significant association was found between presence of lymph node metastasis and stage of the disease. High levels of VEGF staining was seen to correlate with poor disease-specific survival ($p<0.0001$). Thus, VEGF expression in colorectal cancer seems to be an independent prognostic factor and helps to identify patients with unfavourable clinical outcome.

Yong-Song Guan et al., conducted a study in 71 colorectal cancer patients. VEGF expression by the tumor cells had statistically significant association with tumor stage, lymph node and liver metastasis and overall survival.

Lee M. Ellis et al., performed a study in 52 colorectal cancer patients. VEGF expression correlated significantly with presence of lymphovascular invasion and lymph node metastasis. The patients who did not receive any adjuvant chemotherapy were followed up for 5 years and a direct correlation was present between VEGF expression and development of metastatic disease in colorectal cancer ($p<0.0001$)

Qingguo Li et al., conducted a study in 317 colorectal cancer patients. VEGF expression significantly correlated with tumor size, tumor stage, lymph node metastasis and distant metastasis. ($p<0.05$). the 5- year survival rate in patients with positive VEGF expression was 61.9%, whereas it was 70.2% in cases which did not express VEGF.

VEGF

Introduction:

It is a signal protein that stimulates vasculogenesis and angiogenesis[145]. It was discovered in 1983 by Sanger et al. [146]

Functions:

Its normal function is to create new blood vessels during embryonic development, after injury and to bypass blocked vessels. Solid cancers require adequate blood supply to grow beyond a limited size. Cancers expressing VEGF can grow and metastasize.

Subtypes:

In mammals, VEGF comprises of five members, VEGF-A, Placental growth factor (PIGF), VEGF-B, VEGF-C & VEGF-D.

Mechanism of action:

All the members of VEGF family act by binding to tyrosine kinase receptors on the cell surface. They are activated by transphosphorylation. The

hypoxic cells produce HIF (Hypoxia inducible factor) which stimulates the release of VEGF.

VEGF receptors are classified into VEGF-R1, VEGF-R2, VEGF-R3. VEGF-R2 is located in vascular endothelial cells and VEGF-R3 on lymphatic endothelial cells and stimulate vasculogenesis and lymphangiogenesis respectively. Circulating VEGF binds to VEGFR on endothelial cells triggering tyrosine kinase pathway leading to angiogenesis.

Anti-VEGF Therapy:

The first anti-VEGF drug Bevacizumab was approved in 2004. Many Anti-VEGF drugs are under trial.

IMMUNOHISTOCHEMISTRY:

It is a method based on antigen-antibody reaction for localizing specific antigens in tissues or cells.

In 1940, Coons detected antigens in frozen tissue sctions[147]. Taylor and Burns demonstrated antigens in FFPE tissues[148]. The antibody is labeled with an enzyme. Visualization of the labeled antibody by light microscopy is enabled by adding a suitable chromogen substrate. Greater sensitivity is obtained by using detection systems like Avidin-biotin complex, Peroxidase-antiperoxidase, Biotin-Streptavidin method and polymer based labeling systems.

The technique if immunohistochemistry includes the following steps:

- Preparation of adhesive coated slides
- Cutting of 4-5 micron sections
- Deparaffinisation of the sections
- Blocking the endogenous enzymes like peroxidase, alkaline phosphatase to avoid non-specific staining
- Antigen retrieval to unmask the antigen
- various methods can be used for antigen retrieval like use of the water bath, autoclaving, microwave heating or pressure cooker treatment.
- The next step includes blocking of non-specific binding sites
- Binding of primary antibody
- Binding of secondary antibody
- Use of detection methods like peroxidase - antiperoxidase, avidin – biotin conjugates, avidin – streptavidin complexes, polymer based detection systems.
- Addition of chromogen substrate, usually DiAminoBenzidine(DAB)
- Counterstaining, dehydrating and mounting the sections.

Quality control in IHC:

Quality control measures should be taken care of during the pre-analytical, analytical and post – analytical phases.

Use of controls:

Positive control tests the presence of antigen, integrity if the antibody and validates the methodology. The test is run on a tissue known to be immunoreactive for a particular primary antibody. It should have positive staining.

For the purpose of negative control, the same section used for positive control should be used. The primary antibody is replaced by a non-immune antiserum in the same dilution of the primary antibody.

Recent advances in IHC:

GENOGENIC IMMUNOHISTOCHEMISTRY:

It can be used to detect molecular changes by IHC. It can be used for diagnosis and therapy. With regard to colon cancer, microsatellite instability can be detected by genogenic immunohistochemistry.

MONOCLONAL ANTIBODY DEVELOPMENT:

Development of highly specific antibodies using recombinant technology has paved way for the make of molecules with high stability, high potency and ultra-high affinity.

AUTOMATION IN IMMUNOHISTOCHEMISTRY:

Automated techniques are available for the IHC procedure and also for microscopic image analysis. Computerised image capture and analysis systems are available.

MATERIALS AND METHODS

The study is based on colorectal adenocarcinomas. It is a combined prospective and retrospective study conducted in the Institute of Pathology, Madras Medical College, Chennai. It has been conducted over a period of 2 years from July 2013 to July 2015.

We received 147 cases of resected specimens of colorectal carcinomas for histopathological examination in our Institute of Pathology, during the period between July 2013 to July 2015.

Inclusion Criteria:

Colorectal malignancies diagnosed in resected specimens.

Exclusion Criteria:

Small biopsies

Lymphoma of the colon

GIST

Non-neoplastic and benign lesions

Method of data collection:

Relevant clinical details of the patients undergoing surgery for colorectal malignancy, regarding age, gender, procedure done were collected. 4um thick sections were taken from formalin fixed paraffin embedded tissue blocks and stained with hematoxylin and eosin. Among them, 50 random cases were selected for immunohistochemical studies.

Immunohistochemical analysis was done using VEGF.

Variables Studied:

The clinopathological variables studied were, age, gender, site of the tumor, procedure done, size of the tumor, gross appearance, histological type, grading and staging of the tumor, other prognostic factors like lymphovascular invasion and presence of lymph node metastasis.

Representation FFPE tissue samples were subjected to the with VEGF and immunoreactivity was analysed.

Antigen	**Vendor**	**Species (clone)**	**Dilution**	**Positive Control**
VEGF	Pathnsitu	Mouse	Ready to use	Vascular endothelial cells

Immunohistochemistry Procedure:

Slide Preparation:

Sections with a thickness of 4-5u were cut from FFPE tissue and transferred to slides coated with gelatin and chrome alum.

The slides were incubated overnight at 58^{o}C. The sections were deparaffinised in xylene for 30 minute (15 minute x 2 changes)

The sections were dehydrated with absolute alcohol for 10 minutes (5 minutes x 2 changes). The sections were then rinsed in distilled water for 5 minutes.

Antigen Retrieval

Retrieval buffer was prepared and was preheated for 4 minutes at 800W.

The sections were then immersed in the retrieval buffer and incubated in the oven at 800W (5 minutes), 640W (5 minutes x 2 times) and 480W (5 minutes).

The slides were cooled at room temperature, washed with distilled water and then with TBS for 3 minutes (2 changes).

Peroxidase block was applied over the sections for 10 minutes and then washed with TBS for 2 minutes (2 changes).

Antibody Application:

The sections were treated with primary antibody for 30 minutes and washed with TBS for 2 minutes (2 changes).

They were treated with polyexcel target binder for 15 minutes and washed with TBS for 2 minutes (2 changes).

The sections were treated with HRP for 15 minutes and washed with TBS for 2 minutes (2 changes).

Chromogen Application:

DAB substrate was prepared by diluting 1 drop of DAB chromogen to 1ml DAB buffer. DAB substrate solution was applied on the sections for 5 minutes.

The slides were washed with distilled water for 2 minutes.

The sections were counterstained with hematoxylin for 10 seconds.

The slides were washed with distilled water for 5 minutes, air dried, cleared with xylene and mounted with DPX.

Interpretation and scoring system:

The slides were analysed for the presence of immunohistochemical reaction, percentage of cells stained and intensity of the reaction.

The slides were screened for cytoplasmic positivity of VEGF. Intensity of staining was classified as weak, moderate and strong.

Scoring was done based on the proportion of the cells stained.

0 - no tumor cells show positivity

1 - <10 tumor cells show cytoplasmic positivity

2 - 11-50% cells show cytoplasmic positivity

3 - >50% cells show cytoplasmic positivity

Statistical analysis:

The statistical analysis was done using Statistical Package for Social Science software. The expression of VEGF was correlated with variables like age, gender, location, size, grade, stage, histological grade, lymph node involvement and lymphovascular invasion.

OBSERVATION AND RESULTS

From July 2013 to July 2015, we received 147 resected specimens of colorectal cancer for histopathological examination in the Institute of Pathology, Madras Medical College.

Age distribution of the study participants: (Table 1, Fig. 1)

In this study the median age at presentation of colorectal cancer is 60 years. The youngest age of presentation is 23 years and the oldest age of presentation is 79 years. Most of the cases were in the age range of 51 to 60 years (42.9%).

Table 1: Age distribution among the cases

Age range	Frequency	Percent	Valid Percent	Cumulative Percent
<30	4	2.7	2.7	2.7
31-40	18	12.2	12.2	15.0
41-50	26	17.7	17.7	32.7
51-60	63	42.9	42.9	75.5
61-70	24	16.3	16.3	91.8
>70	12	8.2	8.2	100.0
Total	147	100.0	100.0	

Gender wise distribution of colorectal cancer: (Table 2, Fig. 2)

In this study the incidence of colorectal cancer showed a male preponderance. Out of 147 cases 85 (57.8%) were males and 62 (42.2%) cases were females.

Table 2: Gender distribution among the tumors

Gender	Frequency	Percent	Valid Percent	Cumulative Percent
Males	85	57.8	57.8	57.8
Females	62	42.2	42.2	100.0
Total	147	100.0	100.0	

Site distribution of colorectal cancer: (Table 3, Fig.3)

Out of 147 cases, 104 (70.7%) cases occurred in left colon, 43 (29.3%) cases occurred in the right colon. Thus, in this study, left sided tumors were found to be more common than right sided tumors.

Table 3: Distribution of side among the tumors

Side	Frequency	Percent	Valid Percent	Cumulative Percent
Right	43	29.3	29.3	29.3
Left	104	70.7	70.7	100.0
Total	147	100.0	100.0	

Distribution of colorectal cancer based on size: (Table 4, Fig. 4)

Out of 147 cases, the tumor was $\leq$ 5cm in 79(54%) cases and >5cm in 68(46%) cases

Table 4: Distribution of size among the tumor

size	Frequency	Percent
>5	68	46.3
<5	79	53.7
Total	147	100.0

Distribution of colorectal cancer based on macroscopic appearance:

(Table5, Fig 5)

Out of 147 cases. 92 (62.6%) cases were ulceroproliferative 23(15.6%) were ulcerative, 12 (8.2%) were ulceronodular, 14(9.5%) were circumferential. 2 (1.4%) cases presented as stricture and 4 (2.7%) cases presented with polypoidal masses.

Table 5: Distribution of gross features among the tumors

Gross	Frequency	Percent	Valid Percent	Cumulative Percent
Ulceroprliferative	92	62.6	62.6	62.6
Ulcerative	23	15.6	15.6	78.2
Ulceronodular	12	8.2	8.2	86.4
Circumferential	14	9.5	9.5	95.9
Stricture	2	1.4	1.4	97.3
Polypoidal	4	2.7	2.7	100.0
Total	147	100.0	100.0	

Distribution of colorectal cancer based on histopathological subtypes : (Table 6, Fig. 6)

Among the 147 cases, 123 (83.7%) were infiltrating adenocarcinoma, 6(4.1%) were infiltrating adenocarcinoma with mucinous differentiation 14(9.5%) were mucinous carcinoma. 3(2%) were signet ring cell carcinoma, 1(0.7%) was adeno carcinoma with neuroendocrine differentiation.

Table 6: Distribution of microscopic types among the cases

Microscopic subtype	Frequency	Percent	Valid Percent	Cumulative Percent
Infiltrating adenocarcinoma	123	83.7	83.7	83.7
Adenocarcinoma with mucinous diff.	6	4.1	4.1	87.8
Mucinous carcinoma	14	9.5	9.5	97.3
Signet ring cell carcinoma	3	2.0	2.0	99.3
Adeno ca with neuroendocrine diff.	1	.7	.7	100.0
Total	147	100.0	100.0	

Grade wise distribution of colorectal cancer: (Table 7, Fig. 7)

Out of 147 cases of colorectal cancer, 37(25.2%) well differentiated, 95(64.6%) were moderately differentiated and 15(10.2%) were poorly differentiated.

Table 7: Distribution of grade among the tumors

Grade of differentiation	Frequency	Percent	Valid Percent	Cumulative Percent
Well	37	25.2	25.2	25.2
Moderate	95	64.6	64.6	89.8
Poor	15	10.2	10.2	100.0
Total	147	100.0	100.0	

Stage wise distribution of colorectal cancer: (Table 8, Fig 8)

Among 147 cases, 36 (24.5%) cases presented in stage B1, 44(29.9%) in B2, 14(9.5%) in C1 and 53 (36.1%) cases in C2. Most of the cases were in the stage C2.

Table 8: Distribution of stage among the cases

Stage	Frequency	Percent	Valid Percent	Cumulative Percent
B1	36	24.5	24.5	24.5
B2	44	29.9	29.9	54.4
C1	14	9.5	9.5	63.9
C2	53	36.1	36.1	100.0
Total	147	100.0	100.0	

Distribution of cases based on lymph node involvement: (Table 9, Fig.9)

Among the 147 cases studied, 66(44.9%) had lymph node involvement and in 81 (55.1%) cases, there was no involvement of lymph nodes.

Table 9: Distribution of cases based on lymph node involvement

Lymph node involvement	Frequency	Percent	Valid Percent	Cumulative Percent
Absent	81	55.1	55.1	55.1
Present	66	44.9	44.9	100.0
Total	147	100.0	100.0	

Distribution of other prognostic factors in colorectal cancer:

Out of the 147 cases, lymphatic invasion was present in 74 (50.3%) cases (Table 10, Fig.10), vascular invasion was present in 67 (45.6%)cases(Table 11,Fig.11), lymphocytic response to the tumor was present in 76 (51.7%) cases(Table 12,Fig 12). Resected margins were free of tumor in 143 (97.3%) cases(Table 13, Fig 13).

Table 10: Distribution of lymphatic invasion among the cases

Lymphatic invasion		Frequency	Percent	Valid Percent	Cumulative Percent
	Absent	73	49.7	49.7	49.7
	Present	74	50.3	50.3	100.0
	Total	147	100.0	100.0	

Table 11: Distribution of vascular invasion among the cases

Vascular invasion	Frequency	Percent	Valid Percent	Cumulative Percent
Absent	80	54.4	54.4	54.4
Present	67	45.6	45.6	100.0
Total	147	100.0	100.0	

Table 12: Distribution of lymphocytic response among the tumor

Lymphocytic response	Frequency	Percent	Valid Percent	Cumulative Percent
Absent	71	48.3	48.3	48.3
Present	76	51.7	51.7	100.0
Total	147	100.0	100.0	

Table 13: Margin status of the tumors

Margin status	Frequency	Percent	Valid Percent	Cumulative Percent
Free	143	97.3	97.3	97.3
Involved	4	2.7	2.7	100.0
Total	147	100.0	100.0	

RESULTS OF IMMUNOHISTOCHEMICAL ANALYSIS:

Out of the 147 cases, 50 random cases were selected and subjected to immunohistochemical analysis for VEGF expression.

Among the 50 cases, 9 cases(18%) showed cytoplasmic VEGF expression in <10 % of tumor cells(1+), 20 cases(40%) showed positivity in 10-50% tumor cells(2+) and 21 cases(42%) expressed cytoplasmic VEGF in >50% tumor cells(3+).(Table14, Fig. 14)

Table 14: Distribution of varying levels of VEGF expression among the cases

VEGF expression	Frequency	Percent	Valid Percent	Cumulative Percent
1+	9	18.0	18.0	18.0
2+	20	40.0	40.0	58.0
3+	21	42.0	42.0	100.0
Total	50	100.0	100.0	

CORRELATION OF VEGF EXPRESSION WITH VARIOUS CLINICOPATHOLOGICAL PARAMETERS:

AGE:

Among the 50 cases chosen, youngest and oldest ages were 28 and 79 years respectively. Median age for 1+, 2+ and 3+ VEGF expression were 57, 57 and 59 years respectively. The association between VEGF expression and age is statistically insignificant. (Table 15, Fig.15)

Table 15: Correlation of age and VEGF expression

Correlation of age and VEGF expression			Mean	Median	Minimum	Maximum	Standard Deviation	Standard Error of Mean
VEGF	1+	AGE	57.56	57.00	47.00	65.00	6.56	2.19
	2+	AGE	57.15	57.00	28.00	79.00	13.98	3.13
	3+	AGE	59.10	59.00	33.00	78.00	11.32	2.47

GENDER:

Among the 50 cases, 25 were males and 25 were females. Out of the 25 males 1+, 2+ and 3+ VEGF expression were found in 4,9 and 12 patients respectively.

Among the 25 females, 1+, 2+ and 3+ VEGF expression were found in 5,11 and 9 cases respectively. The association between gender and VEGF expression is statistically insignificant.(Table 16)

Table 16: Correlation between gender and VEGF expression

Correlation between gender and VEGF expression			VEGF			Total
			1+	2+	3+	
Gender	Male	Count	4	9	12	25
		% within VEGF	44.4%	45.0%	57.1%	50.0%
	Female	Count	5	11	9	25
		% within VEGF	55.6%	55.0%	42.9%	50.0%
	Total	Count	9	20	21	50
		% within VEGF	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=.740 p>0.05(0.691)

LOCATION:

Out of the 50 cases chosen 14 were right sided tumors and 36 were left sided tumors. Among the right sided tumors, 1+,2+ and 3+ VEGF expression was found in 4,7 and 3 cases respectively. Out of the 36 left sided tumors , 1+,

2+ and 3+ VEGF expression were found in 5,13 and 18 cases respectively(Table 17). The association between VEGF expression and location of the tumor is statistically insignificant.

Table 17: Correlation between location of the tumor and VEGF expression

Correlation between VEGF expression and location of the tumor		**VEGF**			**Total**
		1+	**2+**	**3+**	
Right	Count	4	7	3	14
	% within VEGF	44.4%	35.0%	14.3%	28.0%
Left	Count	5	13	18	36
	% within VEGF	55.6%	65.0%	85.7%	72.0%
Total	Cont	9	20	21	50
	% within VEGF	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=3.653 p>0.05(0.161)

SIZE:

Of the 50 cases chosen, 25 were less than 5 cm and 25 were more than 5 cm in greatest dimension(Table18). The association between VEGF expression and size of the tumor is statistically insignificant.

Table 18: Correlation between tumor size and VEGF expression

Correlation between size of the tumor and VEGF expression		VEGF			Total
		1+	2+	3+	
SIZE	<5 cm Count	3	13	9	25
	<5 cm % within VEGF	33.3%	65.0%	42.9%	50.0%
	>5 cm Count	6	7	12	25
	>5 cm % within VEGF	66.7%	35.0%	57.1%	50.0%
Total	Count	9	20	21	50
Total	% within VEGF	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=3.229 p>0.05 (0.199)

GRADE:

Among the 50 cases, 16 were well differentiated, 29 were moderately differentiated and 5 were poorly differentiated. The varying levels of VEGF expression by these tumors is shown in Table 19 . The association between VEGF expression and grade of the tumor is statistically insignificant.

Table 19: Correlation between VEGF expression and grade of the tumor

Correlation between VEGF expression and grade of the tumor		VEGF			Total
		1+	2+	3+	
Well	Count	2	7	7	16
	% within VEGF	22.2%	35.0%	33.3%	32.0%
Moderate	Count	7	12	10	29
	% within VEGF	77.8%	60.0%	47.6%	58.0%
Poor	Count	0	1	4	5
	% within VEGF	0.0%	5.0%	19.0%	10.0%
Total	Count	9	20	21	50
	% within VEGF	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=4.467 p>0.05(0.347)

LYMPHATIC INVASION:

Out of the 50 cases, 35 cases had lymphatic invasion and 2,14 ,19 cases had 1+,2+,3+ VEGF expression respectively(Table20,Fig 16). The association between VEGF expression and presence of lymphatic invasion proved to be statistically significant.

Table 20 : Correlation between VEGF expression and presence of lymphatic invasion

Correlation between VEGF expression and lymphatic invasion		VEGF			Total
		1+	2+	3+	
Absent	Count	7	6	2	15
	% within VEGF	77.8%	30.0%	9.5%	30.0%
Present	Count	2	14	19	35
	% within VEGF	22.2%	70.0%	90.5%	70.0%
Total	Count	9	20	21	50
	% within VEGF	100.0%	100.0%	100.0%	100.0%

$p<0.05$

VASCULAR INVASION:

Among the 50 cases, 35 cases had vascular invasion and 3,15,17 cases had 1+,2+,3+ VEGF expression respectively(Table 21, Fig 17). The association between VEGF expression and presence of vascular invasion proved to be statistically significant.

Table 21: Correlation between VEGF expression and presence of vascular invasion

Vascular invasion	**VEGF**			
	1+	**2+**	**3+**	**TOTAL**
Absent	6	5	4	15
%	67%	25%	19%	30%
Present	3	15	17	35
%	33%	75%	81%	70%
Total	9	20	21	50
	18%	40%	42%	
	CHI SQUARE	7.20	p value =	0.027

LYMPH NODE METASTASIS:

Out of the 50 cases, 27 patients had metastasis to the regional lymph nodes; 1,10,16 cases had 1+,2+,3+ VEGF expression respectively(Table 22, Fig 18). The association between VEGF expression and presence of lymph node metastasis is statistically significant.

Table 22: Correlation between VEGF expression and lymph node involvement

Correlation between VEGF expression and lymph node involvement			VEGF			Total
			1+	2+	3+	
	Absent	Count	8	10	5	23
		% within VEGF	88.9%	50.0%	23.8%	46.0%
	Present	Count	1	10	16	27
		% within VEGF	11.1%	50.0%	76.2%	54.0%
Total		Count	9	20	21	50
		% within VEGF	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=10.956* $p<0.05$ significant

STAGE:

Among the 50 cases chosen, 5,18,8,19 cases belonged Astler-Coller stage B1,B2,C1,C2 respectively(Table 23, Fig 19). Varying levels of VEGF expression in these tumors is given in Table The association between VEGF expression and stage of the tumor is found to be statistically significant.

Table 23: Correlation between VEGF expression and stage of the tumor

Correlation between VEGF expression and stage			VEGF			Total
			1+	2+	3+	
Stage	B1	Count	3	1	1	5
		% within VEGF	33.3%	5.0%	4.8%	10.0%
	B2	Count	5	9	4	18
		% within VEGF	55.6%	45.0%	19.0%	36.0%
	C1	Count	1	5	2	8
		% within VEGF	11.1%	25.0%	9.5%	16.0%
	C2	Count	0	5	14	19
		% within VEGF	0.0%	25.0%	66.7%	38.0%
Total		Count	9	20	21	50
		% within VEGF	100.0%	100.0%	100.0%	100.0%

Pearson Chi-Square=19.607* $p<0.05$ significant

The overall clinicopathological profile is given in Table 24.

Clinico-pathological factor		**No. of cases (%)**
Age	<50 years	48(32.7%)
	>50 years	99(67.3%)
Histological type	Infiltrating adenocarcinoma	123(83.7%)
	Infiltrating adenocarcinoma with mucinous differentiation	6(4.1%)
	Mucinous adenocarcinoma	14(9.5%)
	Signet ring cell adenocarcinoma	3(2%)
	Infiltrating adenocarcinoma with neuroendocrine differentiation	1(0.7%)
Grade of differentiation	Well	37(25.2%)
	Moderate	95(64.6%)
	Poor	15(10.2%)

Clinico-pathological factor		No. of cases (%)
Stage	B1	36(24.5%)
	B2	44(29.9%)
	C1	14(9.5%)
	C2	53(36.1%)
Lymph node	Positive	66(44.9%)
	Negative	81(55.1%)
Tumor Size	<=5 cm	79(54%)
	>5 cm	68(46%)
VEGF expression	1+	9(18%)
	2+	20(40%)
	3+	21(42%)

DISCUSSION

Globally, colorectal cancer poses a major public health problem. It is the fourth most common cause of death from cancer, worldwide. In India, cancer of the colon ranks among the top ten in men and women.

Survival is mainly dependant on the stage of the disease at diagnosis; with a better 5-year survival for patients diagnosed at the localized stage. Many biological markers are being studied, in order to explore their prognostic significance.

In this study, Immunohistochemical evaluation is done in 50 cases of colorectal carcinoma and is correlated with various clinicopathological parameters.

The median age of presentation of colorectal carcinoma is 60 years; the youngest and oldest ages of presentation being 23 and 79 years respectively.

COMPARISON WITH OTHER STUDIES MEDIAN AGE

Table 25 : Comparison of median age with other studies

Studies	**Troisi et al.[150]**	**Robin.P.Burshey et al.[151]**	**Chu KC et al.[152]**	**Gendi et al[153]**	**Current study**
Median age (yrs)	60	72	58	55	60

The median age of occurrence of colorectal carcinoma is compared with other studies.

Troisi et al. conducted a study in 223 colorectal carcinoma patients and found that the mean age at diagnosis was 60 years.

Robin.P.Burshey et al. conducted a study in 168 colorectal carcinoma patients and concluded that the median age at diagnosis was 72 years.

Chu KC et al. and Gendi et al conducted studies in 108 and 159 colorectal carcinoma patients and found that the median age at diagnosis was 58 and 55 years respectively.

The median age at diagnosis of colorectal carcinoma in this study is 60 years which is in concurrence with the stuby done by Troisi et al. (Table 25)

LOCATION OF THE TUMOR

Table 26: Comparison of tumor location with other studies

Tumor location	Left side	Right side
Troisi et al[150]	52.5%	47.5%
Kazem et al [155]	56.7%	43.3%
N.Scott et al [154]	69.2%	30.8%
Gendi et al[153]	75%	25%
Current study	70.7%	29.3%

In a study conducted by Kazem et al. and Scott et al ,left sided tumors constituted about 56.7%, 69.2% and right sided constituted about 43.3%,308% respectively.

In the current study, left and right sided tumors constitute about 70.7% and 29.3% respectively, which is in concurrence with the study conducted by Gendi et al ., in which left sided tumors were about 75% and right sided tumors were about 25% (Table 26).

HISTOLOGICAL TYPES

Table 27: Comparison of histological subtype of colorectal carcinoma with another study

Histological type	Kazem et al[159]	Current Study
Infiltrating Adenocarcinoma	86.7%	83.7%
Infiltrating Adenocarcinoma With Mucinous Differentiation	-	4.1%
Signet Ring Cell Carcinoma	3.3%	2%
Mucinous Adenocarcinoma	10%	19.5%
Adenocarcinoma with neuroendocrine differentiation	-	0.7%

In the study conducted by Kazem et al. in 323 colorectal carcinoma patients, the most common microscopic subtype was infiltrating adenocarcinoma which constituted about 86.7%, followed by mucinous carcinoma which was about 10% The main histolopathological subtype of colorectal carcinoma in this study is Infiltrating adenocarcinoma which is in concurrence with the study of Kazem et al. (Table 27)

COMPARISON OF EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND CLINICOPATHOLOGICAL CORRELATION IN COLORECTAL CARCINOMA WITH OTHER STUDIES:

Association between presence of lymphatic and vascular invasion and VEGF expression:

In the current study, the association between VEGF expression and the presence of lymphovascular invasion is clinically significant. This is in concurrence with many studies conducted elsewhere. (Table 28)

Table 28: Comparison of VEGF expression and presence of lymphovascular invasion with other studies

Studies	No. of cases studied	p Value for lymphovascular invasion and VEGF expression
Yukata et al.	52	0.03
Kang SM et al	156	0.004
Tucker et al.	27	0.014
Current study	50	0.027

In a study conducted by Yukata et al in 52 colorectal carcinoma patients, significant statistical association was found between presence of lymphovascular invasion and lymph node involvement. Also in studies conducted by Kang SM et al. and Tucker et al. in 156 and 27 patients respectively, there was a significant association between VEGF expression and presence of lymphatic and vascular invasion.

Table 29: Comparison of VEGF expression and presence of lymph node involvement with other studies

Studies	No. of patients studied	p value for lymph node involvement and VEGF expression
Martius et al[159]	672	0.03
Yeb CYet al[160]	58	0.042
Wang D et al[161]	317	0.039
Lee JC et al[162]	92	0.042
Current study	50	0.04

Association between VEGF expression and lymph node involvement:

In this study, there is a significant association(p value<0.05) between presence of lymph node metastasis and level of VEGF expression.(Table 29). In studies conducted by Martius et al., Yeb CY et al., Wang D et al., and Lee JC et al. in 672, 58, 317 and 92 colorectal carcinoma patients respectively, there is significant association between VEGF expression and lymph node involvement

Association between stage of the disease and VEGF expression:

In this study, there is a significant association between staging of the tumor and levels of VEGF expression. Tumors belonging to Astler-Coller stage C2 had expressed more VEGF when compared to others. (Table 30)

Table 30: Comparison of VEGF expression and tumor stage with other studies

Studies	No. of cases studied	p Value for stage of the disease and VEGF expression
Bendarafa et al[163]	360	0.04
Okita et al[164]	91	0.032
Ochs et al[165]	109	0.04
Zafirellis et al[166]	117	0.026
Current study	50	0.038

Also in studies conducted by Okita et al., Bendarafa et al., Ochs et al., Zafirellis et al., in 91, 360, 109 and 117 colorectal carcinoma patients, significant statistical association was found between VEGF expression and stage of the tumor.

CORRELATION BETWEEN TUMOR DIFFERENTIATION AND VEGF EXPRESSION:

Table 31: comparison of VEGF expression and tumor differentiation with other studies

Studies	Tumor differention	VEGF 1+	VEGF 2+	VEGF 3+	p value
Shu Zheng et al.	Well Moderate Poor	10 5 3	6 11 9	12 20 21	0.028
Current study	Well Moderate Poor	2 7 0	7 12 1	7 10 4	0.34

In the current study, 16 cases were well differentiated tumors. Out of the 16 cases, 1+, 2+ and 3+ VEGF expression was found in 2,7 and 7 cases respectively. There were 29 moderately differentiated tumors, among which 1+, 2+ and 3+ VEGF expression were found in 7, 12 and 10 cases respectively. Out of the 5 poorly differentiated tumors, 1+, 2+ and 3+ VEGF expression were found in 0,1 and 4 cases respectively. There is no significant association between grade of the tumor and VEGF expression.

In a study conducted by Shu Zheng et al., in 97 colorectal cancer patients, there was a significant statistical association between VEGF expression and grade of the tumor.

Qingguo et al., conducted a study in 317 colorectal cancer patients, there was no significant association between VEGF expression and tumor differentiation.

SUMMARY

From July 2013 to July 2015, 147 resected specimens of colorectal cancer were received for histopathological examination in Institute of Pathology, Madras Medical College.

- The median age at presentation is 60 years
- Youngest age of presentation is 23 years
- Oldest age of presentation is 79 years
- Maximum number of cases occurred in the age group of 50-60 years
- There is a male preponderance -85 cases(57.8%)
- Left sided tumors are more common-104 cases(70.7%)
- 54% cases had tumors less than 5 cm in greatest dimension
- Commonest gross appearance is ulceroproliferative -92 cases(62.6%) followed by the ulcerative type-23 cases(15.6%)
- Most common histopathological subtype is Infiltrating adenocarcinoma-123 cases(83.7%).
- Commonest grade is moderately differentiated-95 cases(64.6%)
- 53 cases(36.1%) belonged to Astler-Coller stage C2.
- 66 cases(44.9%) had lymph node involvement
- 74 cases(50.3%) had lymphatic invasion
- 67 cases(45.6%) had vascular invasion
- 76 cases(51.7%) had tumor infiltrating lymphocytes
- Resected margins were free in 147(97.3%) cases

- Out of 147 cases, immunohistochemical analysis was done for expression of VEGF in 50 random cases.
- 9(18%), 20(40%) and 21(42%) cases had 1+,2+ and 3+ levels of VEGF expression respectively
- There is no significant association between VEGF expression and factors like age, gender, location, size and grade of the tumor
- Statistically significant association is present between VEGF expression and factors like presence of lymphatic invasion, vascular invasion, lymph node metastasis and stage of the tumor.

CONCLUSION

Among the 147 cases studied at the Institute of Pathology, Madras Medical College from July 2013-July 2015, median age at presentation is 60 years, with a male preponderance. Left sided tumors are common; Infiltrating adenocarcinoma with moderate differentiation being the predominant histopathological subtype.

Immunohistochemical analysis was performed to assess the expression of Vascular Endothelial Growth Factor by the tumor cells in 50 cases. Significant association was found between VEGF expression and stage of the tumor, presence of lymphatic invasion, vascular invasion and lymph node metastasis.

This study throws light on the need for targeted therapy with anti-VEGF drugs to minimize pathological vasculogenesis in the tumor, occurrence of regional and distant metastasis, thereby improving patient survival.

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ANNEXURE I

Bethesda Guidelines for HNPCC (Revised)

1. Colorectal cancer diagnosed before age 50 years
2. Multiple colorectal cancer or HNPCC-related cancers[a]
3. Colorectal cancer with MSI-related histology[b] diagnosed before age 60 years
4. Colorectal cancer or HNPCC-related cancer diagnosed in at least one first-degree relative before age 50 years
5. Colorectal cancer or HNPCC-related cancer diagnosed in at least two first- or second-degree relatives at any age

Any criterion (1 to 5) can be met.

HNPCC, hereditary nonpolyposis colorectal cancer; MSI, microsatellite instability.

[a] Includes cancer of endometrium, small bowel, pelvis/ureter, biliary tract, stomach, ovary, pancreas, or brain (mainly glioblastoma multiforme).

[b] Includes tumor-infiltrating lymphocytes, Crohn-like lymphocytic reaction, mucin/signet ring cell differentiation, and medullary growth pattern.

ANNEXURE II

WHO histological classification of tumours of the colon and rectum

Epithelial tumours

- Adenoma
  - Tubular
  - Villous
  - Tubulovillous
  - Serrated

Intraepithelial neoplasia (dysplasia)

associated with chronic inflammatory diseases

- Low-grade glandular intraepithelial neoplasia
- High-grade glandular intraepithelial neoplasia

Carcinoma

- Adenocarcinoma
- Mucinous adenocarcinoma
- Signet-ring cell carcinoma
- Small cell carcinoma
- Squamous cell carcinoma
- Adenosquamous carcinoma
- Medullary carcinoma
- Undifferentiated carcinoma

Carcinoid (well differentiated endocrine neoplasm)

- EC-cell, serotonin-producing neoplasm
- L-cell, glucagon-like peptide and PP/PYY producing tumour
- Others

Mixed carcinoid-adenocarcinoma

Others

Non-epithelial tumours

Lipoma

Leiomyoma

Gastrointestinal stromal tumour

Leiomyosarcoma

Angiosarcoma

Kaposi sarcoma

Malignant melanoma

Others

Malignant lymphomas

- Marginal zone B-cell lymphoma of MALT Type
- Mantle cell lymphoma
- Diffuse large B-cell lymphoma
- Burkitt lymphoma
- Burkitt-like /atypical Burkitt-lymphoma
- Others

Secondary tumours

Polyps

Hyperplastic (metaplastic)

Peutz-Jeghers

Juvenile

ANNEXURE III

American Joint Committee on Cancer Staging for Colorectal Cancer

American Joint Committee on Cancer	Dukes Staging	Modified Astler-Coller Classification
Stage 0: Tis, N0, M0	A	A
Stage I: T1, N0, M0 or T2, N0, M0	A	A/B_1
Stage IIA: T3, N0, M0	B	B_2
Stage IIB: T4, N0, M0	B	B_2
Stage IIIA: T1-2, N1, M0	C	C_1
Stage IIIB: T3-4, N1, M0	C	C_2
Stage IIIC: T any, N2, M0	C	C_1/C_2
Stage IV: T any, N any, M1	D	D

Tis, carcinoma in situ; T1, tumor invades into submucosa; T2, tumor invades into muscularis propria; T3, tumor invades through the muscularis propria into the subserosa or into nonperitonealized pericolonic or perirectal tissue; T4, tumor perforates the visceral peritoneum or invades directly into other organs or tissues; N0, no lymph node metastases; N1, metastatic tumor in one to three pericolic or perirectal lymph nodes; N2, metastatic tumor in four or more pericolic or perirectal lymph nodes; N3, metastases in any lymph node along the course of a major named blood vessel; M0, no distant metastases; M1, distant metastases present.

KEY TO MASTER CHART

GENDER

1-MALE

2-FEMALE

P/D-PROCEDURE DONE

1-RIGHT HEMICOLECTOMY

2-LEFT HEMICOLECTOMY

3-ABDOMINOPERINEAL RESECTION

4-ANTERIOR RESECTION

5-ULTRA LOW ANTERIOR RESECTION

6- SIGMOIDECTOMY

7-PELVIC EXENTRATION

8-SUBTOTAL COLECTOMY

9-TOTAL PROCTOCOLECTOMY

10-HARTMANN'S PROCEDURE

LOCATION

1-RIGHT

2-LEFT

GROSS APPEARANCE

1-ULCEROPROLIFERATIVE

2-ULCERATIVE

3-ULCERONODULAR

4-CIRCUMFERENTIAL

5-STRICTURE

6-POLYPOIDAL

HPE-HISTOPATHOLOGICAL EXAMINATION

1-INFILTRATING ADENOCARCINOMA

2-INFILTRATING ADENOCARCINOMA WITH MUCINOUS DIFFERENTIATION

3-MUCINOUS CARCINOMA

4-SIGNET RING CELL CARCINOMA

5-INFILTRATING ADENOCARCINOMA WITH NEUROENDOCRINE DIFFERENTIATION

GRADE

1-WELL DIFFERENTIATED

2-MODERATELY DIFFERENTIATED

3-POORLY DIFFERENTIATED

LN METS-LYMPH NODE INVOLVEMENT

Y-PRESENT

N-ABSENT

LI-LYMPHATIC INVASION

Y-PRESENT

N-ABSENT

VI-VASCULAR INVASION

Y-PRESENT

N-ABSENT

LCI-LYMPHOCYTIC INFILTRATION

Y-PRESENT

N-ABSENT

MAC- MODIFIED ASTLER-COLLER STAGE

MARGINS

1-FREE

2-INVOLVED

VEGF EXPRESSION

1+ <10% CELLS SHOW CYTOPLASMIC POSITIVITY

2+ 10-50% CELLS SHOW CYTOPLASMIC POSITIVITY

3+ >50% CELLS SHOW CYTOPLASMIC POSITIVITY

MASTER CHART

S.No	HPE No	Age	Gender	Location	P/D	Size	Gross	HPE	Grade	LCI	LI	VI	LN Mets	MAC	Margins	VEGF
1	2906/13	61	1	2	10	3.5*2*1	1	1	1	N	N	N	N	2	1	
2	2931/13	49	2	2	4	4*4*1.5	1	1	2	N	N	N	N	1	1	
3	3959/13	60	1	2	10	3*2*1	1	1	2	N	Y	Y	Y	4	1	
4	4634/13	55	2	2	10	5*3*3	3	2	3	N	Y	Y	Y	4	1	
5	4644/13	55	2	2	10	2*1*0.5	2	1	2	Y	N	N	N	3	1	
6	5056/13	65	2	2	6	4*3.5*1.5	1	1	2	Y	Y	Y	Y	3	1	
7	5078/13	46	1	2	3	2*2*1.5	1	1	2	Y	N	N	N	1	1	
8	5347/13	60	1	2	10	6*5*4	1	3	2	N	N	N	N	1	1	
9	5582/13	36	1	2	6	3*2*0.5	2	1	2	N	N	N	N	2	1	
10	5611/13	60	1	2	10	7*4*2.5	1	1	2	N	Y	Y	Y	4	2	
11	5814/13	45	2	2	3	6*4*1	2	1	1	N	Y	Y	Y	4	1	
12	5969/13	53	1	2	3	6*4*1.5	1	1	1	N	Y	N	Y	4	1	
13	7235/13	66	1	2	10	3.5*2.5*1	1	1	2	Y	Y	Y	Y	4	1	
14	7424/13	43	1	2	2	10*5*5	1	2	2	Y	N	N	N	2	1	
15	7597/13	51	1	2	3	5*4*2	1	1	2	Y	Y	Y	Y	3	1	
16	7739/13	55	1	2	3	4*4*1	1	1	2	N	N	N	N	1	1	
17	8330/13	29	1	1	1	9*7*4	1	1	2	Y	Y	Y	Y	4	1	
18	8523/13	47	2	2	3	5*4*1	1	1	1	N	N	N	N	1	1	
19	8618/13	56	1	2	3	6*4*2	1	1	2	N	N	N	N	1	1	
20	9552/13	52	1	1	1	10*9*4	1	1	2	Y	Y	Y	Y	4	1	
21	9860/13	50	1	2	4	7*3*1.5	2	1	2	N	Y	N	Y	4	1	
22	10203/13	65	1	1	1	3*2*0.5	1	1	1	N	Y	Y	Y	4	1	
23	10414/13	55	1	2	2	6*5*1	1	1	2	Y	N	N	N	1	1	
24	10507/13	40	2	1	1	4*3*1.5	4	3	2	N	Y	Y	Y	4	1	
25	10641/13	65	1	2	3	5*4*1	1	1	3	N	Y	Y	Y	4	1	
26	11100/13	58	2	1	1	13*10*5	1	2	2	N	N	N	N	1	1	
27	11075/13	55	1	2	9	4*3*2	6	1	1	N	N	N	N	1	1	

S.No	HPE No	Age	Gender	Location	P/D	Size	Gross	HPE	Grade	LCI	LI	VI	LN Mets	MAC	Margins	VEGF
28	11135/13	65	2	1	1	4*2*1	1	1	2	Y	N	N	N	2	1	
29	2689/13	55	2	2	4	1.5*1*1	3	1	2	Y	N	N	N	1	1	
30	2856/13	23	1	1	1	8*6*3	1	4	3	N	Y	Y	Y	4	1	
31	3114/13	35	2	2	4	3*2*1	3	3	2	N	Y	Y	Y	4	1	
32	3194/13	60	1	1	1	10*7*2	1	1	2	Y	N	N	N	1	1	
33	3482/13	46	1	2	4	1.5*1*0.5	3	1	2	N	N	N	N	1	1	
34	3528/13	53	1	2	3	3*2.5*1	2	1	2	N	Y	Y	Y	4	1	
35	4265/13	57	1	1	8	7*5*2	1	1	2	N	N	N	N	2	1	
36	4467/13	75	1	2	3	2*1.5*1	2	1	2	Y	N	N	N	1	1	
37	4938/13	70	1	2	4	2.5*2.5*1	1	1	2	N	Y	Y	Y	4	1	
38	5209/13	45	1	2	4	4*2.5*1	1	1	2	N	Y	Y	Y	4	1	
39	5542/13	33	1	2	3	6*3*1	1	4	2	N	Y	N	Y	4	1	
40	6272/13	40	2	2	7	4*3*1.5	1	1	2	N	N	N	N	2	1	
41	7991/13	34	1	2	2	9*8*3	1	3	2	N	N	N	N	2	1	
42	8208/13	60	1	2	3	5*4*2	1	1	2	Y	N	N	N	1	1	
43	8840/13	28	2	2	4	3.5*3.5*1	1	1	2	N	Y	Y	Y	4	1	
44	10654/13	55	2	2	4	2.5*1*1	1	1	2	Y	N	N	N	1	1	
45	10665/13	62	2	1	1	8*4*4	1	1	2	Y	N	N	N	1	1	
46	11024/13	38	1	2	1	6*3*1	2	1	2	N	N	N	N	1	1	
47	11126/13	53	1	2	7	6*4*2	2	3	2	N	N	N	N	2	1	
48	11203/13	72	1	2	11	7*6*0.5	1	1	2	N	Y	N	Y	3	1	
49	30/14	72	1	1	1	6*6*1	1	5	3	N	Y	Y	Y	4	1	
50	180/14	70	1	2	3	2*1*0.5	3	1	2	N	Y	Y	Y	4	2	
51	1006/14	55	2	1	1	3*1*0.5	5	1	2	Y	N	N	N	2	1	
52	1041/14	52	2	2	3	3*2*1	2	1	2	Y	N	N	N	2	1	
53	740/14	51	2	1	10	3*3*2	1	1	2	Y	Y	Y	Y	3	1	2+
54	869/14	58	1	2	4	3*2*1	2	1	2	N	N	N	N	2	1	

S.No	HPE No	Age	Gender	Location	P/D	Size	Gross	HPE	Grade	LCI	LI	VI	LN Mets	MAC	Margins	VEGF
55	920/14	40	2	2	3	2*2*0.5	1	1	2	N	Y	Y	Y	4	1	
56	1653/14	42	1	1	1	15*10*5	1	1	3	Y	Y	Y	Y	4	1	
57	1695/14	28	2	2	3	4*4*3	1	1	1	Y	Y	Y	Y	4	1	2+
58	1892/14	47	2	2	2	5*2.5*1	1	1	1	Y	N	N	N	1	1	
59	3263/14	58	2	2	3	8*2*1	1	1	1	N	Y	Y	Y	3	1	3+
60	3385/14	70	1	2	3	0.5*0.5*0.5	2	1	1	Y	N	N	N	1	1	
61	4360/14	49	2	2	4	5*3*2	2	1	1	Y	N	N	N	2	1	
62	4417/14	39	2	2	4	8*8*2	1	1	3	N	Y	Y	Y	4	2	
63	4509/14	65	2	2	7	1.5*1*0.5	3	1	2	N	N	N	N	2	1	
64	4810/14	60	1	2	6	9*9*2	1	1	1	Y	N	N	N	2	1	
65	5179/14	59	2	1	1	3*2*1	1	1	2	N	Y	Y	Y	4	1	3+
66	5830/14	47	1	2	5	10*7*1.5	1	1	2	Y	Y	Y	Y	4	1	
67	6069/14	54	1	2	6	8*8*1.5	4	1	1	Y	Y	N	Y	4	1	3+
68	6153/14	60	1	2	3	4*3*1.5	1	1	3	N	Y	Y	Y	4	2	
69	6223/14	58	1	2	3	6*3*1.5	1	1	1	N	Y	Y	Y	3	1	1+
70	6263/14	60	2	1	1	5*3*0.5	1	1	1	Y	N	N	N	1	1	
71	6402/14	55	2	2	3	2*1*0.5	1	1	2	N	Y	Y	Y	3	1	
72	6432/14	56	2	2	3	7*5*3	4	3	2	Y	N	N	N	2	1	
73	6781/14	65	1	2	2	10*10*2	1	3	2	Y	N	Y	N	2	1	1+
74	6878/14	60	1	2	3	3.5*2*1.5	1	1	1	Y	N	N	N	2	1	
75	7249/14	33	1	1	1	5*4*2	4	3	2	N	Y	Y	Y	4	1	2+
76	7310/14	38	1	2	7	6*5*2	2	1	2	N	N	N	N	1	1	
77	7536/14	51	2	2	5	5*4*1	3	1	2	Y	Y	Y	Y	3	1	2+
78	7715/14	59	1	2	3	4*2*1	1	1	1	Y	Y	Y	Y	4	1	3+
79	7935/14	62	1	2	5	5*5*1	3	1	2	Y	Y	Y	Y	3	1	3+
80	8009/14	56	1	2	3	5*5*2	1	1	2	N	N	N	N	2	1	1+
81	8133/14	60	2	2	3	6*3*2.5	1	1	2	N	Y	N	Y	4	1	

S.No	HPE No	Age	Gender	Location	P/D	Size	Gross	HPE	Grade	LCI	LI	VI	LN Mets	MAC	Margins	VEGF
82	8266/14	70	1	2	11	4.5*3*1	4	1	2	Y	Y	Y	Y	4	1	3+
83	8569/14	35	1	1	1	5.5*5*3	2	1	2	N	Y	Y	Y	4	1	
84	8812/14	47	2	1	1	6*3*1	1	1	2	Y	N	Y	N	1	1	1+
85	8928/14	46	1	2	6	3*2*1	2	1	2	N	Y	Y	Y	3	1	2+
86	9173/14	35	1	2	3	2*1*0.5	6	3	2	N	N	N	N	1	1	
87	9256/14	50	2	2	3	7*2*1	4	3	2	N	N	N	N	1	1	
88	9581/14	32	1	1	1	4*3*0.5	2	1	2	Y	N	N	N	1	1	
89	9929/14	43	2	2	2	5*3*1	1	1	2	N	Y	N	Y	4	1	3+
90	10249/14	75	2	1	1	10*6*2	1	1	1	Y	N	N	N	2	1	2+
91	10300/14	60	2	2	6	5*5*1	1	1	2	Y	Y	Y	Y	4	1	3+
92	10829/14	79	2	2	3	3*3*1.5	1	2	1	Y	Y	Y	Y	3	1	2+
93	11193/14	60	1	1	1	17*15*2	1	1	3	N	Y	Y	Y	4	1	3+
94	11429/14	66	2	2	2	6*5*4	4	1	2	Y	N	N	N	2	1	
95	11545/14	57	1	2	3	3*2*1	3	3	2	N	Y	Y	Y	3	1	1+
96	11854/14	45	1	2	2	9*4*2.5	4	1	1	Y	N	N	N	1	1	
97	1252/14	65	2	1	1	8*4*1	1	1	2	Y	N	N	N	2	1	1+
98	1503/14	54	1	1	1	11*8*2	1	1	2	Y	N	N	N	2	1	
99	2659/14	60	2	1	1	2*1*0.5	2	1	2	Y	N	N	N	2	1	
100	2777/14	80	2	2	9	4*4*1	3	1	2	N	Y	N	Y	4	1	
101	3090/14	40	1	1	1	5*3*2	1	3	2	Y	N	N	N	1	1	
102	3199/14	59	2	2	9	7*5*1	1	1	2	Y	N	N	N	1	1	
103	3428/14	58	1	2	3	1*1*0.5	2	1	1	Y	N	N	N	1	1	
104	3871/14	57	1	1	1	6*5*1.5	1	1	1	Y	N	N	N	1	1	
105	4030/14	55	2	2	4	4*3*0.5	1	1	1	Y	Y	Y	N	2	1	3+
106	5307/14	65	2	1	1	5*4*1	1	1	1	Y	N	N	N	1	1	1+
107	5543/14	55	1	2	2	3*3*1	1	1	1	N	Y	Y	Y	4	1	
108	6033/14	46	1	2	3	2*1*0.5	2	1	2	Y	Y	N	Y	4	1	

S.No	HPE No	Age	Gender	Location	P/D	Size	Gross	HPE	Grade	LCI	LI	VI	LN Mets	MAC	Margins	VEGF
109	6113/14	51	1	2	3	1*1*0.5	2	1	2	N	N	N	N	2	1	
110	6384/14	50	1	1	1	6*4*2	1	1	3	Y	N	N	N	2	1	
111	7033/14	66	2	1	1	2*1*0.5	5	1	2	Y	Y	Y	Y	4	1	
112	7809/14	52	2	1	1	7*6*1	1	1	2	Y	N	N	N	2	1	
113	8866/14	76	1	2	11	3*3*0.5	1	1	2	N	Y	Y	N	2	1	2+
114	9754/14	65	2	2	6	4*2*1	2	1	2	N	Y	N	Y	4	1	2+
115	10640/14	62	1	1	1	10*8*1.5	1	1	3	Y	Y	Y	Y	4	1	2+
116	10807/14	60	1	1	1	2*1*0.5	1	1	2	Y	N	N	N	2	1	
117	11316/14	70	1	2	2	5.5*5*1	1	1	2	N	Y	N	N	2	1	2+
118	11984/14	34	2	1	1	12*7*1.5	3	2	1	Y	Y	Y	Y	4	1	
119	1/15	40	2	2	2	14*10*2	4	1	3	N	N	N	N	2	1	
120	640/15	33	2	2	3	10*5.5*3	1	4	3	N	Y	Y	N	2	1	3+
121	919/15	78	1	2	3	5*4*2.5	1	1	2	Y	N	Y	N	2	1	3+
122	1144/15	75	2	2	3	8*5*1	1	1	2	Y	Y	N	N	2	1	3+
123	302/15	42	1	2	3	6*4*1	1	1	3	N	Y	Y	Y	4	1	3+
124	786/15	55	2	2	3	7*3*2.5	1	1	2	Y	N	Y	N	2	1	1+
125	1281/15	73	1	1	1	9*6*4	1	1	2	N	Y	Y	Y	4	1	3+
126	1601/15	75	1	2	4	8*4.5*1	6	1	1	Y	N	N	N	1	1	3+
127	1793/15	56	2	2	3	3*3*0.5	1	1	2	Y	Y	Y	Y	3	1	2+
128	2057/15	61	1	2	3	2*2*0.5	3	1	2	Y	Y	Y	N	2	1	2+
129	2224/15	46	1	2	3	9*9*1	1	1	1	Y	N	N	N	1	1	
130	2230/15	75	2	2	6	5*5*1	1	1	1	N	N	Y	N	2	1	2+
131	2461/15	55	2	2	9	4*3*1.5	6	1	2	N	Y	N	Y	4	1	3+
132	2557/15	55	1	1	1	10*8*3	1	1	1	Y	N	N	N	1	1	
133	1578/15	57	2	1	1	3*2.5*1	2	1	1	Y	N	N	N	2	1	
134	1673/15	43	1	1	1	8*5*5	1	1	1	Y	N	N	N	2	1	
135	1875/15	49	2	1	1	5*3*1	4	3	2	Y	N	N	N	2	1	

S.No	HPE No	Age	Gender	Location	P/D	Size	Gross	HPE	Grade	LCI	LI	VI	LN Mets	MAC	Margins	VEGF
136	2120/15	63	1	2	3	7*6*2	1	1	3	Y	Y	Y	Y	4	1	
137	2546/15	60	2	2	3	6*4*2	1	1	2	Y	N	Y	N	2	1	2+
138	2815/15	51	1	2	11	3*2*1	4	1	2	Y	N	Y	N	2	1	2+
139	2819/15	50	2	1	1	10*8*6	4	2	2	Y	Y	N	N	1	1	1+
140	2895/15	55	2	2	10	10*5*2	1	1	3	N	Y	Y	Y	4	1	3+
141	1578/15	57	2	1	1	3*2.5*1	4	1	1	N	N	N	N	2	1	2+
142	4737/15	44	2	1	1	4*3*1	1	1	1	Y	N	Y	N	2	1	2+
143	3857/15	60	1	2	3	6*5*1	1	1	1	N	Y	Y	Y	4	1	3+
144	4371/15	63	1	2	3	3.5*3*1	4	1	2	Y	Y	Y	Y	4	1	3+
145	2224/15	46	1	2	4	9*9*1	1	1	1	Y	Y	Y	N	1	1	2+
146	4368/15	52	1	2	3	6.5*5*1	2	1	1	N	Y	Y	Y	4	1	3+
147	4742/15	57	1	1	1	6*5*1	1	3	2	N	Y	N	Y	4	1	2+